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RESEARCH ARTICLE

ANABOLIC-ANDROGENIC STEROID (STANOZOLOL) DISRUPTS OVARIAN HISTOARCHITECTURE AND GESTATION IN MICE

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ABSTRACT

In the past decades, the therapeutic use of anabolic androgenic steroids (AAS) has been overshadowed by illicit abuse of these drugs by athletes and non-athletes. Consequently AAS can have adverse effect like coronary heart diseases, reproductive and endocrine disturbances. The present investigation was undertaken to determine the efficacy of one of the AASs stanozolol on the maintenance of pregnancy in Swiss albino mice. Normal pregnant mice were assigned to three experimental groups. Two groups received stanozolol through sc. (0.5mg/kg bwt/day in 3% alcohol) from day 8 through 14 or 19 of pregnancy. Control animals received vehicle alone (3% alcohol). The results revealed that treatment of stanozolol is not able to maintain gestation to full term, resulting in partial fetal resorption with many placentomas, placental scars and no viable fetuses indicate the functional failure of corpus luteum. Histomorphology and differential follicular count revealed a noticeable decrease in secondary and antral follicles, increase in atretic follicles and an insignificant reduction in the number of corpora lutea in both treatment groups. It is concluded that stanozolol interrupts pregnancy and its effect may be mediated through reduction in the developing follicles and principally due to the deficiency of luteal hormones.

INTRODUCTION

Anabolic-androgenic steroids (AAS) are a group of synthetic derivatives of testosterone. They were designed to provide enhanced anabolic potency with negligible androgenic effects (Jorge-Rivera *et al*., 2000), that are now being used clinically for the treatment of endometrosis, aplastic anemia, male hypogonadism and inoperable breast cancer (rev: Kochakian, 1993). In addition to legitimate therapeutic applications, these AAS compounds are being abused by many recreational and professional athletes to enhance the muscle mass, athletic performance, physique, strength and endurance (Lukas, 1993; Wilson, 1988). However, the adverse physiological effects of AAS abuse are largely unknown. Besides, in recent years the use of AAS is becoming increasingly popular among the adolescent girls and women athletes; and the actual influence of these compounds on overall physiology, hypothalamo-hypophysial-gonadal axis is yet to be established clearly. In this context, we wish to know the role of one of the AAS compounds stanozolol (17α-alkylated compound which is a derivative of dihydroxy testosterone) on histoarchitecture of the ovary and gestation in mice. In a previous experiment, we have noted that stanozolol accelerates granulopoiesis and stimulates immune response (at physiologic level only), though it alters the lipoprotein profile in mice (Inamdar and Jayamma, 2012). Pregnancy is an anabolic state associated with increased maternal metabolic activity, where in the ovarian and adrenal steroids play a vital role (Venning, 1946, Heap, 1972). The pituitary plays an indispensable role and its hormones are necessary part of the luteotropic complex during the first 10 days of gestation. Thereafter, hormones from the placenta take over the role of the pituitary hormones. As a consequence placental androgens secreted into the maternal circulation serve as precursors for luteal estrogen production, so the level of estrogen does not decline and it continues to act locally to promote luteal structure and function which is essential at midpregnancy (rev: Gibori *et al*., 1988, Bazer, 1999; Spencer, 1999). In this perspective the present investigation examines the efficacy of AAS compound stanozolol (17α-alkylated compound which is a derivative of Dehydro-testosterone) in the maintenance of the pregnancy in mice with following objectives:

1. to know the effect of stanozolol on maintenance of pregnancy.
2. to investigate its effect on gravimetric and histoarchitecture of the ovary .
3. to examine the effect of this compound on the fetal survival rate.

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MATERIALS AND METHODS

Normal adult female mice (*Mus musculus*) of Swiss albino 'strains' were obtained from the mice colony maintained in the Department of Zoology, Karnatak University, Dharwad. They were housed in individual cages at room temperature (27±1ºC), with natural cum-artificial light for 12-14h and fed with a pelleted diet (Goldmohur, lipton, India) and water *ad libitum*. Female mice exhibiting normal estrous cycles and weighing 25-30 gm (3-months-old) were mated with fertile males at proestrus or early estrus phase. The mice showing sperm in their vaginal smear on the following morning were selected for experiment with that day being designated as day 1 of pregnancy. In the present experiment one of the AAS compounds stanozolol [(17β-hydroxy-17α-methyl-androstano (3, 2-c) pyrazole from Sigma Chemical Co., St. Louis, MO] of which a therapeutic dose of 0.5mg/kg bwt was administered by s.c. injection in 3% alcohol to pregnant mice (n= 5) from day 8 to 14, and day 8 to 19 (n=5). The pregnant mice (n=5) that received 3% alcohol served as controls. Body weight recorded daily. On day 20 of pregnancy, all mice were autopsied by giving pentobarbitone sodium (40mg/kg bwt) subcutaneous injection prior to anesthesia. The ovaries, uterus with fetuses, and placenta, thymus were dissected out, freed from connective tissue and weighed. The number of live and dead fetuses, placentomas, and placental scars were recorded (Fig. 7). Ovaries from 5 animals in each group were fixed in Bouin's fluid and processed for standard histological procedures. Serial sections of the ovaries (5µm thick) stained with Harris' hematoxylin eosin were subjected for observation ((Nikon microscope 90E with ACT 2U software).

Follicular kinetics

Differential follicular count was made from every 10th section of the ovary (Fig. 8). For this purpose ovarian follicles were classified based on the methodology opted by Pederson and Peters (1968) and Hirshfield and Midgley (1978).

Statistical analysis

Data were expressed as Mean ±S.E. Comparisons of normally distributed variables across groups were calculated using one-way ANOVA followed by Post hoc test (LSD). The level of statistical significance was set at $P<0.05$ and $P<0.01$. All the analyses were performed using SPSS (version 10.0 for windows) and recorded values are summarized in Figs. 7, 8 and Table 1.

RESULTS

It has been observed that in control pregnant mice the pregnancy was maintained to full term with 100 % survival of fetuses (Fig. 1). Treatment of stanozolol interrupted pregnancy in both the treated groups. Treatment of this AAS compound to pregnant mice from day 8 to 14 and day 8 to 19 resulted in fetal resorptions with placentomas and many placental scars (Figs. 2 and 3) which were noticed on the day of autopsy. Slight vaginal bleeding was observed in these mice on day 10 and day 14 of pregnancy in mice that received drug from day 8 through14 while vaginal bleeding was observed on day 15 of pregnancy for day 8 to 19 treatment groups. As implantation was not affected on the day of autopsy the total number of placentomas and placental scars were taken into account, which revealed that treatment of stanozolol resulted in the increased number of placentomas and placental scars ($F_{2,14}=35.6$; $P<0.01$) with no live fetuses in both the treatment groups when compared to control (Fig. 7**).**

Histoarchitecture and morphometry of the ovary

Histomorphology of the ovaries in control pregnant mice revealed the peripheral region/ cortex consisting of less number of developing follicles (DF), more corpus luteum (CL) and few atretic follicles (AF) were found embedded in comparatively scanty stromal tissue formed by richly vascularized loose connective tissue (Fig. 4). The corpus luteum (CL) was large in size with big lutenised cells possessing distinct vesicular nuclei and rich cytoplasm. Treatment of stanozolol from day 8 to 14 of pregnancy revealed an insignificant decrease in the diameter of the ovary, while in the other treatment group (day 8 to 19) an increase in the ovarian diameter ($F_{2,14}=1.3$; NS) was observed when compared control (Fig.5 & 6).

Table I. Effect of stanozolol on body and organ weights in pregnant albino mice

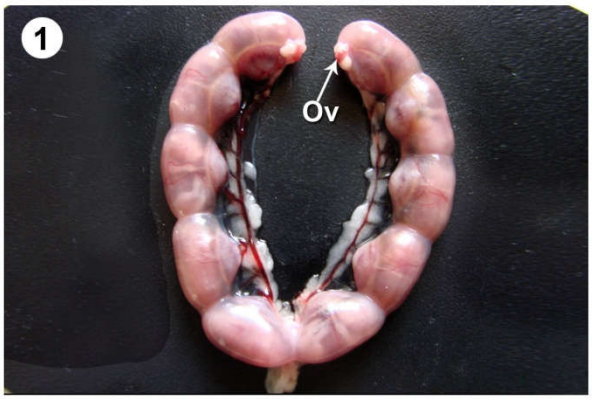
Sl.no.	Observations	Control 0.5ml of 3% alcohol. n=5	Day-8 to 14 of pregnancy. Stanozolol- 0.5 mg/kg/bwt n=5	Day-8 to 19 of Pregnancy. Stanozolol -0.5mg/kg n=5	F value	P value
1	Body wt. (gm)	61.4±1.7	36.8±0.5	36.8±0.02	149.8	<0.01
2	Ovary wt. (gm)	0.0095±4.5	0.0082±0.00097	0.0132±0.00153	6.2	<0.05
3	Uterus wt. (gm)	11.4±0.2	0.2±0.03	0.2±0.03	4315.4	<0.01
4	Adrenal wt. (gm)	0.009±0.0003	0.007±0.0007	0.0052±0.0004	14.0	<0.01
5	Thymus wt. (gm)	0.2±0.09	0.03±0.005	0.05±0.004	1.8	NS

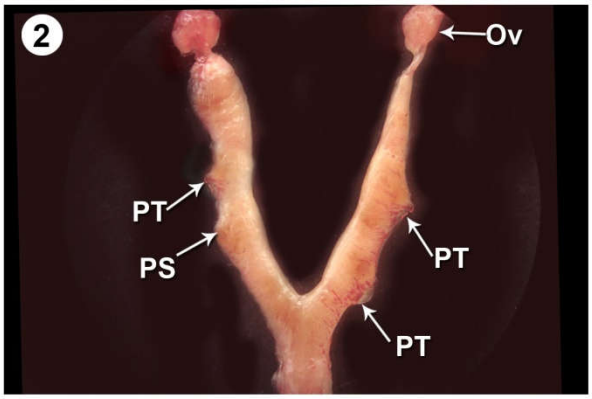
Duration- Day 8 to 14 and 8 to 19 day of pregnancy, Values are expressed as mean±S.E n= number of mice

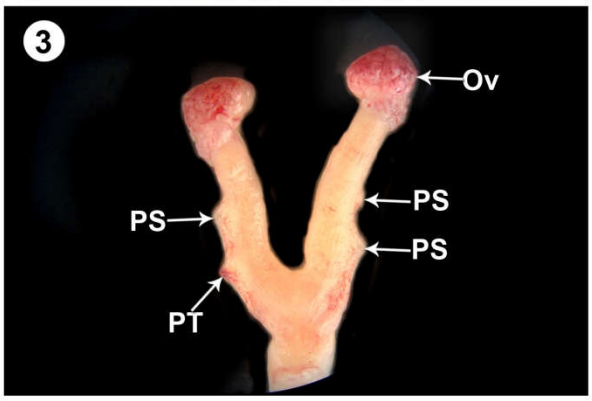
Differential follicular count implies an insignificant decrease in the number of primordial ($F_{2,14}=0.2$; NS), primary ($F_{2,14}=1.4$; NS) and tertiary follicles ($F_{2,14}=1.6$; NS) when compared to control. However, an apparent reduction in the number of secondary ($F_{2,14}=25.9$; $P<0.01$),antral ($F_{2,14}=30.0$; $P<0.01$) and atretic follicles ($F_{2,14}=4.2$; $P<0.05$) was discerned in both the treatment groups when compared to control group (Fig. 8). No appreciable change in the number of Graafian ($F_{2,14}=0.3$; NS) and cystic ($F_{2,14}=2.9$; NS) follicles was observed in both the treatment groups. An insignificant decrease in the number of corpora lutea in day 8 to 14 treatment group and a significant decrease in day 8 to 19 treatment group ($F_{2,14}=17.3$; $P<0.01$) indicates the negative effect of stanozolol on functioning of CL (Fig. 8) Ovarian stroma was found to be increased in stanozolol treated mice from day 8 to 19 day of pregnancy (Fig. 6).

Effect of Stanozolol on body and organ weights

In control mice where the pregnancy was maintained, a significant increase in body weight with a fetal survival of 100% was observed.





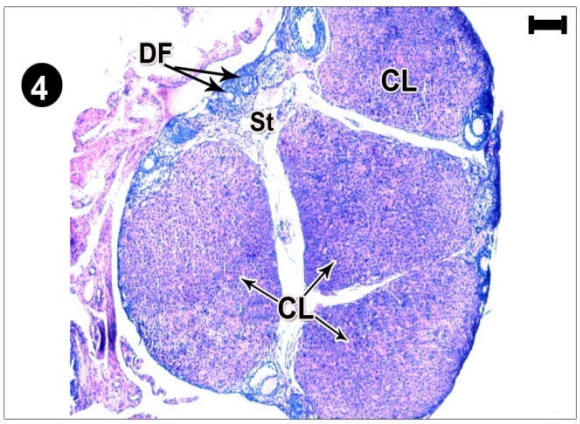


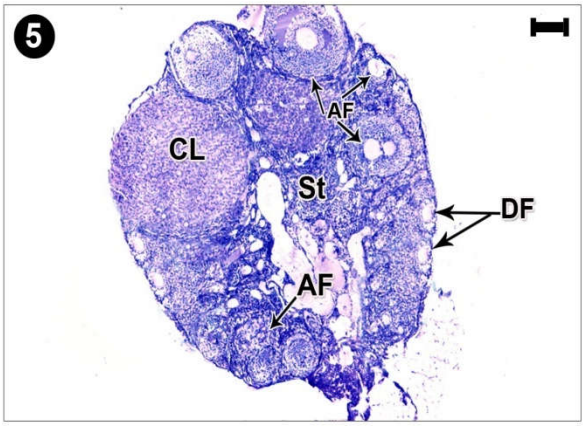
Explanation to Figs. 1-3

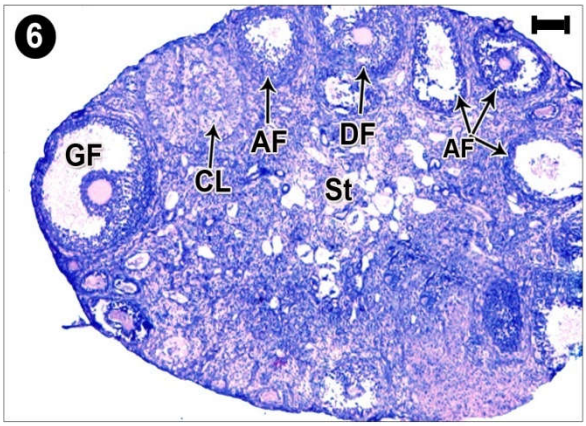
Treatment of stanozolol (0.5mg/kg bwt) to mice from day 8 through 14 or 19 of pregnancy:

1. Uterus of control mouse exhibiting pups which indicates normal pregnancy. 1.7 X. O = Ovary.
2. Treatment of stanozolol to mice from day 8 to 14 of pregnancy and autopsied on day 20 of gestation, revealing partial resorptions as uterus exhibits many placentomas (PT) and placental scars (PS). 1.7 X. O = Ovary.
3. Uterus of stanozolol treated mouse from day 8 to 19 of pregnancy and autopsied on day 20 of gestation, exhibiting many placental scars (PS), placentomas (PT) and absence of viable fetus indicates the interruption of pregnancy. 1.7 X. O = Ovary.

Whereas in stanozolol (0.5 mg/bwt) treated groups from day 8 to 14 and from day 8 to 19 of pregnancy resulted in a considerable reduction in body weight ($F_{2,14}$=149.8; P<0.01) with no live fetal survival was noticed ($F_{2,14}$=135.6; P<0.01). This reduction in body weight in treated mice may be due to abortions or fetal resorptions (Table – 1). Uterine weight was significantly decreased in both the treated groups ($F_{2,14}$=4315.4, P<0.01) when compared to control. An insignificant decrease in the ovarian weight of day 8 to 14 pregnancy and a significant increase ($F_{2,14}$=6.2,







Explanation to Figs. 4-6

Treatment of stanozolol (0.5mg/kg bwt) to mice from day 8 through 14 or 19 of pregnancy:

4. T.S. of the ovary of control pregnant mouse showing normal sized, well developed large corpus luteum (CL), a few developing follicles (DF) with scanty ovarian stroma (St).Scale line- 100µm.
5. T.S. of the ovary of pregnant mouse treated with stanozolol from day 8 to 14 of pregnancy a few developing follicles (DF) and many atretic follicles (AF). Note a decline in the number of corpora lutea (CL) also indicates functional failure of CL.Scale line- 100µm.
6. T.S. of the ovary of pregnant mouse treated with stanozolol from day 8 to 19 of pregnancy showing a few developing follicles (DF), many atretic follicles (AF), bulky ovarian stroma (St) and a regressing Graafian follicle (GF) indicating the deleterious effect of stanazolol on the ovary. Also Note a negligible number of corpora lutea (CL). Scale line-100µm.

P<0.05) in day 8 to 19 pregnancy was observed. No obvious change in thymus weight ($F_{2,14}$=1.8, NS) while a noticeable ($F_{2,14}$=14, P<0.01) decrease in adrenal weight was observed (Table – 1).

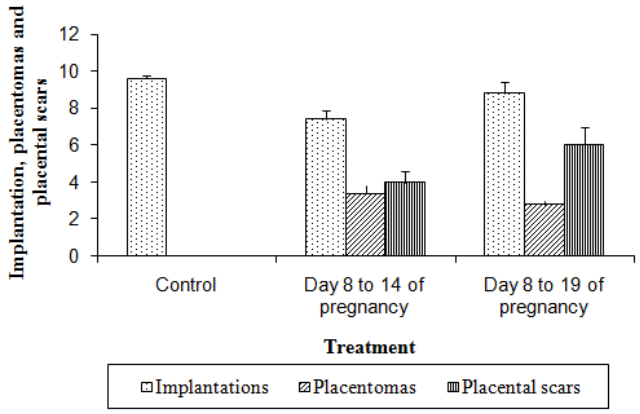


Fig. 7. Treatment of stanozolol to mice from day 8 through 14 or 19 of pregnancy is not able to maintain gestation to full term, resulting in partial fetal resorption with many placentomas and placental scars; no viable fetuses are noticed, indicating the interruption of pregnancy.

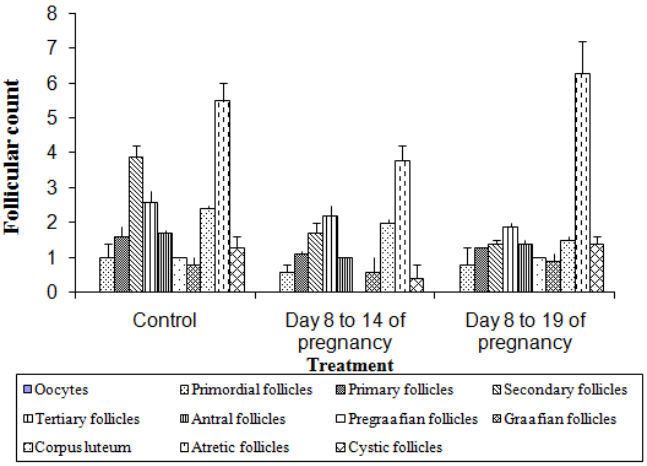


Fig. 8. Differential follicular count of stanozolol treated mice from day 8 through 14 or 19 of pregnancy implies a decrease in the number of primordial, secondary, tertiary antral and atretic follicles follicles in both the treatment groups when compared to control. Also note noticeable decrease in the number of corpora lutea indicating the negative effect of stanozolol on the ovary especially on the functioning of corpora lutea.

DISCUSSION

Successful pregnancy in most mammalian species requires progesterone production by the corpus luteum (CL) (Bazer *et al*., 1991). The function of CL in rodents is regulated by a luteotropic hormonal complex. The Prolactin (PRL) or PRL-like hormone (Lactogen) is one component of the lutetropic complex that is essential for maintaining the functions of the CL such as the progesterone production (rev: Heap, 1972; Richards, 1994; Bazer, 1999; Spencer, 1999). The pituitary plays an indispensable role, particularly in the early part (first half) of the pregnancy, which is mediated through the hypothalamo-hypophyseal-gonadal-adrenal-placental axis (Stucki and Forbes 1960, Bearn *et al*., 1960, Brann *et al*., 1990, Bazer *et al*., 1991, Straus *et al*., 1995, Straus *et al*., 1995, Mastorakos and Ilias 2003). Pituitary, ovarian, fetal and placental interrelationships throughout mouse and rat gestation are complex and not yet fully understood.

In the present study, we investigated the efficacy of stanozolol in the maintenance of pregnancy in mice. It is noted that the administration of 0.5 mg/kg bwt.of this AAS compound from day 8 to 14 and day 8 to 19 of pregnancy cannot maintain gestation to full term. In these treated mice, a slight vaginal bleeding was observed on day 10 and 14 (8 to 14 day treated group) and on day 15 (day 8 to 19 treated group) of pregnancy. Upon autopsy on day 20 of pregnancy, however, the uterus exhibited partial resorptions with many placentomas and placental scars suggest the loss of CL function. In most mammalian species during pregnancy, activated CL secrete progesterone for 12-14 days. After day 12 of pregnancy, the rodent placenta can take over pituitary gonadotrophic function. Although the rodent placenta does not secrete significant amount of progesterone or estrogen, fetal trophoblast cells acquire the capacity to secrete androgens serving as precursors of estrogens for continued leuteotropic support of the corpus luteum at midpregnancy. Hence, the placental androgens secreted into the maternal circulation serve as precurosors for luteal estrogen production, so the level of estrogen does not decline and it continues to act locally to promote CL structure and function (Mastorakos and Ilias 2003, Greenwald and Johnson 1968, Yoshinaga *et al*., 1972; Bazer *et al*., 1991). Further, it is already noted that in rodents, the placenta and deciduas produce prolactin and placental lactogens, respectively, which exert luteotrophic signals to the corpus luteum and ensure production of progesterone during the middle and later stages of pregnancy to term (Bazer 1999; Spencer, 1999). The observed results in the present study suggest that the stanozolol might have a deleterious effect on the placenta as this AAS compound is nonaromatizable androgen and does not serve as precursors for luteal estrogen production; consequently leading to the decline in estrogen and progesterone production resulting in the interruption of pregnancy. In the current experiment a significant reduction in the number of corpora lutea, secondary and antral follicles was observed when compared to control. This reduction is more prominent in mice that received treatment from day 8 through 19 of pregnancy. The reduction in the said follicles, presence of placental scars and absence of viable fetuses, suggest that treatment of stanozolol (though it is of therapeutic dose) may affect the secretion and release of FSH, LH causing an imbalance in progesterone and estrogen secretions by the ovary leading to the interruption of pregnancy. Also the reduction in body weight and uterine weight in treated mice when compared to control is due to abortions or fetal resorptions. Similarly, in an another study it has also been shown that administration of dihydrotestosterone (DHT) suppresses serum progesterone concentration and it exhibits a potent luteolytic activity in the pregnant rats for which authors speculates that this effect is mediated by suppressing the decidual luteotropin (Sridaran and Gibori 1981, 1986). In contrast it has been shown that androstenedione interferes in luteal regression by inhibiting apoptosis and stimulating progesterone production (Goyeneche *et al*., 2002). In another study when pregnant monkeys were given a series of injections of testosterone propionate, three female offspring were pseudohermsphrodites (Van Wagenen and Hamilton 1943). In a recent study on the effect of androgen (testosterone propionate-TP) on implantation and decidualization in the mouse (delayed-implantaion model) reveals that the high dose of TP may disturb peri-implantation development or may be involved in

early pregnancy loss by disturbing the uterine prostaglandin system (Diao *et al*., 2008). On the whole these studies reveal that the different androgens have differential interruptive effect on pregnancy.

The observed results of the present experiment suggest that treatment of stanozolol (though it is of therapeutic dose) may affect the secretion and release of FSH, LH causing an imbalance in progesterone and estrogen secretions by the ovary thus leading to disruption of pregnancy. In conclusion the present study suggests that stanozolol interrupt pregnancy and its effect may be mediated through reduction in the developing follicles and principally due to the deficiency of luteal hormones.

Acknowledgment

All procedures used in this experiment adhere to the CPCSEAguidelines for the Care and Use of Laboratory Animals approved by the Institutional Animal Care and Use Committee No. 639/02 at Department of Zoology, Karnatak University, Dharwad. The authors thank UGC for the financial assistance (MRP(S)-031/07-08/KAKA060/UGC-SWRO) and one of the authors (YJ) is thankful to the UGC for a fellowship under FIP.

REFERENCES

Bazer, F.W. 1999. Pregnancy, maintenance of. In: Knobil E, Neill JD (Eds) Encyclopedia of Reproduction, Vol 3. New York, NY, USA: Academic press., 3:1002-1006.

Bazer, F.W., Simmen, R.C., Simmen, F.A. 1991. Comparative aspects of conceptus signals for maternal recognition of pregnancy. Ann NY Acad Sci., 622: 202-211.

Bearn, J.G., Gould, R.P., Jones, HEH. 1960. The response of adrenal gland of the rat to pregnancy and lactation. Acta Anat., 41: 273.

Brann, D.W., Caria, D., Putrum and Mahesh, V.B. 1990. Corticosteroid regulation of gonadotrophin and prolactin secretion in the rat. Endocrinology 126:159-166.

Diao, H.L., Su, R.W., Tan, H.N., Li, S.J., Lei, W., Deng, W.B., Yang, Z.M. 2008. Effects of androgen on embryo implantation in the mouse delayed-implantation model. Fertil. Steril., 90(4): 1376-1383.

Gibori, G., Khan, I., Warshaw, M.L., McLean, M.P., Puryear, T.K., Nelson, S., Durkee, T.J., Azhar, S., Steinschneider, A., Rao, M.C. 1988. Placental-derived regulators and the complex control of luteal cell function. Recent Prog Horm Res., 44: 377-429.

Goyeneche, A. A., Calvo, V., Gibori, G. and Telleria, C. M. 2002. Androstenedione interferes in luteal regression by inhibiting apoptosis and stimulating progesterone production. Biol. Reprod., 66: 1540-1547.

Greenwald, G. S. and Johnson, D. C. 1968. Gonadotrophic requirements for the maintenance of pregnancy in the hypophysectomized rats. Endocrinology 83:1052.

Heap, R. B. 1972. Role of hormones in pregnancy. In: Hormones in Reproduction. Austin CR, Short RV (Eds) Cambridge Univ. Press, New York, pp. 73-105.

Hirshfield, A. N. and Midgley, A. R. 1978. Morphometric analysis of follicular development in the rat. Biol. Reprod., 19: 597-605.

Inamdar, Laxmi S. (Doddamani) and Y. Jayamma, Y. (2012) Acceleration of neutrophil precursors' maturation and immunostimulation of CD3+, CD4+ lymphocytes by stanozolol in mice. *J. Steroid Biochem. Molec. Biol.,* 129: 172– 178.

Jorge-Rivera, J. C., Mclntyre, K. L. and Henderson, L. P. 2000. Anabolic steroids induce region and subunit-specific rapid modulation of GABA-A receptor mediated currents in the rat Fore brain. J. Neurophysiol., 83: 3299-3309.

Kochakian, C. D. 1993. Anabolic-androgenic steroids: A historical perspective and definition, in: Yesalis, C. E (eds) Anabolic steroids in sport and exercise. Champaign, IL: Human Kinetics Publishers, Inc., 3-33.

Lukas, S. E. 1993. Current perspectives on anabolic androgenic steroid abuse. Trends Pharmaco Sci., 14: 61-68.

Mastorakos, G. and Ilias, I. 2003. Maternal and fetal hypothalamic-pituitary-adrenal axis during pregnancy and postpartum. Ann NY Acad Sci., 997: 136-149.

Pedersen, T. and Peters, H. 1968. Proposal for a classification of oocytes and follicles in the mouse ovary. J. Reprod. Fert., 17: 555-557.

Richards, J. S. 1994. Hormonal control of gene expression in the ovary. Endocr. Rev. 15: 725-751.

Spencer, T. E. 1999. Pregnancy, maternal recognition of. In: Knobil E, Neill JD (Eds) Encyclopedia of Reproduction, Vol 3. New York, NY, USA: Academic press; 1006-1015.

Sridaran, R. and Gibori, G. 1981. Induction of luteolysis by dihydrotestosterone in the pregnant rat. Am J Physiol., 241(6): E444-448.

Sridaran, R. and Gibori, G. 1986. Effects of dihydrotestosterone on progesterone secretion in pseudopregnant rats. Proc Soc Exp Biol Med., 183(3): 328-332.

Straus, J. F. III, Gafvells, M. E., King, B. F. 1995. Placental hormones. In: Degroot LJ (Eds) Endocrinology, 3rd ed. Vol 3. London, Uk: W. B.. Saunders Company. 2171-2206.

Stucki, J. C., Forbes, A. D. 1960. Pregnancy maintenance and the inhibition of Parturition in rats with 17α-hydroxy-progesterone esters: the role of oestrogenic and androgenic activity. Acta Endocr., 33:73.

VanWagenen, G. and Hamilton, J. B. 1943. The experimental production of pseudohermaphroditism in the monkey. In "Essays in Biology", Univ. California Press, Berkeley, California, pp. 583-607.

Venning, E. H. 1946. Adrenal function in pregnancy. Endocrinology 39: 203-230.

Wilson, J. D. 1988. Androgen abuse by athletes. Endocr. Rev. 9: 181-199.

Yoshinaga, K., McDonald, G. J., Greep, R. O. 1972. Influence of various doses of LH on fetal survival in hypophysectomized rats. Proc Soc Exp Biol Med., 140: 893.

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